

WHAT IS CLAIMED IS:

1. A method of creating a nucleic acid multiplex, said method comprising the steps of:

5 1) creating a mixture comprising water, a Watson-Crick duplex, a sufficient number of single-stranded mixed base sequence molecules to form a multiplex that includes the Watson-Crick duplex, and an accelerator agent that increases a rate or amount of multiplex formation, said multiplex being a triplex or quadruplex, wherein said single-stranded molecule or molecules
10 are selected so that, if in a multiplex, they would each be related to all other strands of the multiplex by adherence to base pairing rules, said rules being either Watson-Crick base-pairing rules or homologous binding base-pairing rules; and

2) incubating said mixture to allow the multiplex to form, each strand of said multiplex related to all other strands of the multiplex by adherence to base-pairing rules;

provided that, within the multiplex, the Watson-Crick duplex added in step (1) is heteropolymeric with a G-C content between 10% and 90%.

2. A method of Claim 1 wherein the multiplex created is a triplex, in step (1) the sufficient number of single-stranded molecules is 1, and in step (2) the triplex is formed.

3. A method of Claim 1 wherein the duplex substantially retains its double-helical structure and the single-stranded
25 molecule resides in a groove of that double-helical structure.

4. A method of Claim 3 wherein the single-stranded molecule is related to one strand of the duplex by Watson-Crick base-pairing rules and to the second strand of the duplex by homologous binding base-pairing rules.

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5. A method of Claim 4 wherein the duplex substantially retains its double-helical structure and the single-stranded molecule resides in a groove of that double-helical structure.

5 6. A method of Claim 1 where, within the multiplex, the Watson-Crick duplex added in step (1) is heteropolymeric with a G-C content between 10% and 90%, and furthermore the combined frequencies therein of purine-pyrimidine dimers and pyrimidine-purine dimers exceeds 25%.

10 7. A method of Claim 1 wherein steps (1) and (2) are performed with the nucleic acid strands and/or duplexes not in a cell.

8. A method of Claim 1 wherein step (2) is performed without the assistance of a protein.

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9. A method of Claim 1 wherein in step (1), the water is added so that it accounts, on a volume basis, for at least 50 percent of the final volume of the mixture.

10. A method of Claim 1 wherein in step (1), the water is added so that it accounts, on a volume basis, for at least 80 percent of the final volume of the mixture.

20 11. A method of Claim 1 wherein in step (1), the water is added so that it accounts, on a volume basis, for all of the liquid added to the mixture.

25 12. A method of Claim 1 wherein step (2) is performed at a temperature or temperatures above the freezing temperature of the aqueous solution and at not more than 85 °C.

13. A method of Claim 12 wherein step (2) is performed at a temperature or temperatures between 5 °C and 30 °C.

14. A method of Claim 13 wherein step (2) is performed at a temperature or temperatures between 15 °C and 25 °C.

5 15. A method of Claim 1 wherein in step (1), a cation is added as the accelerator agent.

16. A method of Claim 15 wherein said cation is Na⁺ provided at a concentration of 50mM to 125mM.

sub C 17. A method of Claim 15 wherein said cation is selected from the group consisting of Mn⁺² provided at a concentration of 10mM to 45mM, Mg⁺² provided at a concentration of 10mM to 45mM, and Ni⁺² provided at a concentration of 20mM.

18. A method of Claim 1 wherein in step (1) an intercalator is added as an accelerator agent.

19. A method of Claim 18 wherein the intercalator is a fluorescent intercalator.

20 20. A method of Claim 19 wherein the fluorescent intercalator is selected from the group consisting of YOYO-1, TOTO-1, YOYO-3, TOTO-3, POPO-1, BOBO-1, POPO-3, BOBO-3, LOLO-1, JOJO-1, cyanine dimers, YO-PRO-1, TO-PRO-1, YO-PRO-3, TO-PRO-3, TO-PRO-5, PO-PRO-1, BO-PRO-1, PO-PRO-3, BO-PRO-3, LO-PRO-1, JO-PRO-1, cyanine monomers, ethidium bromide, ethidium homodimer-1, ethidium homodimer-2, ethidium derivatives, acridine, acridine orange, acridine derivatives, ethidium-acridine heterodimer, 25 ethidium monoazide, propidium iodide, SYTO dyes, SYBR Green 1, SYBR dyes, Pico Green, SYTOX dyes, and 7-aminoactinomycin D.

21. The method of Claim 1 wherein the accelerator agent is a non-intercalating fluorophore.

22. A method of Claim 21 wherein the non-intercalating fluorophore is selected from the group consisting of biotin, rhodamine, Alexa dyes, BODIPY dyes, biotin conjugates, thiol-reactive probes, fluorescein and derivatives including but not limited to the caged probes, Oregon Green, Rhodamine Green, QSY dyes.

23. A method of Claim 1 wherein in step (1) the accelerator agent is an intercalator that binds to the minor and/or major groove of the Watson-Crick duplex.

24. The method of Claim 1 wherein in step (1) the accelerator agent at 25 °C is a liquid.

25. The method of Claim 24 wherein in step (1) the accelerator agent is an organic liquid soluble in water.

26. The method of Claim 1 wherein in step (1) an accelerator agent that is a condensation agent as regards the Watson-Crick duplex is added.

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~~26~~. The method of Claim 1 wherein in step (1) an accelerator agent that is a decondensation agent as regards the Watson-Crick duplex is added.

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~~27~~. A method of detecting a triplex, said method comprising the method of Claim 2 and further comprising an additional step (3) in which the triplex is detected.

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28. A method of Claim 1 wherein the multiplex created is a quadruplex, in step (1) the Watson-Crick duplex is a first Watson-Crick duplex, and in step (1) the sufficient number of single-stranded molecules is 2, those single-stranded molecules are in a second Watson-Crick duplex, and in step (2) the quadruplex is formed from said first and second duplexes. Preferably step (1) is done with the two single-stranded molecules already in the second Watson-Crick duplex.

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29. A method of detecting a quadruplex, said method comprising the method of Claim 28 and further comprising an additional step (3) in which the quadruplex is detected.

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30. A triplex comprising a single-stranded probe bound to a double-stranded nucleic acid target, wherein said probe comprises a heteropolymeric nucleic acid or a heteropolymeric nucleic acid analog, and all base triplets of said triplex are members selected from the group consisting of A-T-A, T-A-T, U-A-T, T-A-U, A-U-A, U-A-U, G-C-G and C-G-C.

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31. A triplex of Claim 30 wherein the double-stranded nucleic acid target substantially retains its double-helical structure and the heteropolymeric nucleic acid or analog resides in a groove of that double-helical structure.

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32. A triplex of Claim 30 wherein the heteropolymeric nucleic acid and analog each have a G-C content between 10% and 90%.

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33. A triplex of Claim 32 wherein the double-stranded nucleic acid target substantially retains its double-helical structure and the heteropolymeric nucleic acid or analog resides in a groove of that double-helical structure.

~~34~~ A quadruplex comprising:

- 35 a first strand containing a first sequence of nucleobases;
a second strand containing a second sequence of nucleobases,
wherein said second strand is associated with said
first strand by Watson-Crick bonding;
a third strand containing a third sequence of nucleobases;
and
a fourth strand containing a fourth sequence of nucleobases,
wherein said fourth strand is associated with said
third strand by Watson-Crick bonding.

36 35. A quadruplex of Claim 34 wherein each of said four
strands is heteropolymeric with a G-C content between 10% and
90%.

37 36. A quadruplex of Claim 34 wherein either (1) the second
and fourth strands are aligned in a parallel 3' to 5' direction
and binding between those 2 strands is according to homologous
base-pairing rules or (2) the first and third strands are aligned
in a parallel 5' to 3' direction and binding between those 2
strands is according to homologous base-pairing rules or (3) the
second and fourth strands are aligned in a parallel 3' to 5'
direction and binding between said second and fourth strands is
according to homologous base-pairing rules and furthermore the
first and third strands are aligned in a parallel 5' to 3'
direction and binding between said first and third strands is
according to homologous base-pairing rules.

38 37. A quadruplex of Claim 36 wherein each of said four
strands is heteropolymeric with a G-C content between 10% and
90%.

38 A quadruplex of Claim 34 wherein either (1) the second and fourth strands are aligned in a parallel 3' to 5' direction and binding between those 2 strands is according to Watson-Crick base-pairing rules or (2) the first and third strands are aligned in a parallel 5' to 3' direction and binding between those 2 strands is according to Watson-Crick base-pairing rules or (3) the second and fourth strands are aligned in a parallel 3' to 5' direction and binding between said second and fourth strands is according to Watson-Crick base-pairing rules and furthermore the first and third strands are aligned in a parallel 5' to 3' direction and binding between said first and third strands is according to Watson-Crick base-pairing rules.

38 A quadruplex of Claim 38 wherein each of said four strands is heteropolymeric with a G-C content between 10% and 90%.

38 A quadruplex of Claim 34 wherein either (1) the first and fourth strands are aligned in anti-parallel 5' to 3' and 3' to 5' directions, respectively, and binding between the 2 strands is according to Watson-Crick base-pairing rules or (2) the second and third strands are aligned in anti-parallel 3' to 5' and 5' to 3' directions, respectively, and binding between those 2 strands is according to Watson-Crick base-pairing rules or (3) the first and fourth strands are aligned in anti-parallel 5' to 3' and 3' to 5' directions, respectively, and binding between said first and fourth strands is according to Watson-Crick base-pairing rules and furthermore the second and third strands are aligned in anti-parallel 3' to 5' and 5' to 3' directions, respectively, and binding between said second and third strands is according to Watson-Crick base-pairing rules.

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41. A quadruplex of Claim 40 wherein each of said four strands is heteropolymeric with a G-C content between 10% and 90%.

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42. A quadruplex of Claim 34 wherein either (1) the first and fourth strands are aligned in anti-parallel 5' to 3' and 3' to 5' directions, respectively, and binding between those 2 strands is according to homologous base-pairing rules or (2) the second and third strands are aligned in anti-parallel 3' to 5' and 5' to 3' directions, respectively, and binding between those 2 strands is according to homologous base-pairing rules or (3) the first and fourth strands are aligned in anti-parallel 5' to 3' and 3' to 5' directions, respectively, and binding between said first and fourth strands is according to homologous base-pairing rules and furthermore the second and third strands are aligned in anti-parallel 3' to 5' and 5' to 3' directions, respectively, and binding between said second and third strands is according to homologous base-pairing rules.

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43. A quadruplex of Claim 42 wherein each of said four strands is heteropolymeric with a G-C content between 10% and 90%.

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44. The quadruplex of Claim 34 wherein each interacting base of the said first strand interacts specifically with both the adjacent base on the said third strand and with the base on the said fourth strand, the base to which the said third strand base is bound.

45.6 A quadruplex of Claim 44 wherein each of said four strands is heteropolymeric with a G-C content between 10% and 90%.

47. The quadruplex of Claim 34 wherein each interacting base of the said second strand interacts specifically with both the adjacent base on the said fourth strand and the base on the said third strand, the base to which the said fourth strand base is bound.

48. A quadruplex of Claim 46 wherein each of said four strands is heteropolymeric with a G-C content between 10% and 90%.

49. The method of Claim 2, said method further comprising a step (3) in which the triplex is detected.

50. The method of Claim 28, said method further comprising an additional step (3) in which the quadruplex is detected.

51. The method of Claim 48, wherein said method discriminates between a perfect base-pairing-rules match, a one-base mismatch or deletion, and a 2-base mismatch or deletion, between the duplex and the single-stranded molecule in the triplex.

52. The method of Claim 49, wherein said method discriminates between a perfect base-pairing-rules match, a one-base mismatch or deletion, and a 2-base mismatch or deletion, between the first and second Watson-Crick duplexes.

53. A method of detecting a triplex, said method comprising:

providing a target double-stranded nucleic acid or nucleic acid analogue comprising a target sequence, wherein said target sequence contains at least one purine base and at least one pyrimidine base;

5 providing a probe comprising a nucleic acid sequence or a nucleic acid analog sequence;

providing an accelerator agent;

10 adding said probe, said target sequence and said accelerator agent to a medium to provide a test sample containing a triplex complex comprising said probe bound to said target sequence, wherein all base triplets of said complex are members selected from the group consisting of A-T-A, T-A-T, U-A-T, T-A-U, A-U-A, U-A-U, G-C-G and C-G-C;

15 irradiating said test sample with exciting radiation to cause the test sample to emit fluorescent radiation;

20 detecting an intensity of said fluorescent radiation, wherein said intensity is correlated with a binding affinity between said probe and said target sequence; and

determining from said intensity an extent of matching between said probe and said target sequence;

25 wherein said method is a homogeneous assay conducted without providing a signal quenching agent on said target sequence or on said probe.

30 ⁵⁴ 53. A method of detecting a triplex, said method comprising: providing a target nucleic acid or nucleic acid analogue having a target sequence, wherein said target sequence contains at least one purine base and at least one pyrimidine base;

providing a double-stranded probe comprising a nucleic acid sequence or a nucleic acid analog sequence;

providing a hybridization accelerator agent;
adding said probe, said target and said hybridization
accelerator agent to a medium to provide a test sample
containing a Watson-Crick triplex comprising said probe
bound to said target sequence;
irradiating said test sample with exciting radiation to
cause test sample to emit fluorescent radiation;
detecting an intensity of said fluorescent radiation,
wherein said intensity is correlated with a binding
affinity between said probe and said target sequence;
and

determining from said intensity an extent of matching
between said probe and said target sequence

wherein said method is a homogeneous assay conducted without
providing a signal quenching agent on said target sequence or on
said probe.

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54. A method of detecting a quadruplex, said method
comprising:

providing a target nucleic acid or nucleic acid analogue
having a target sequence, wherein said target sequence
contains at least one purine base and at least one
pyrimidine base;

providing a double-stranded probe comprising a nucleic acid
sequence or a nucleic acid analog sequence;

providing a hybridization accelerator agent;

adding said probe, said target and said hybridization
accelerator agent to a medium to provide a test sample
containing a Watson-Crick quadruplex comprising said
probe bound to said target sequence;

irradiating said test sample with exciting radiation to
cause test sample to emit fluorescent radiation;

detecting an intensity of said fluorescent radiation,
wherein said intensity is correlated with a binding
affinity between said probe and said target sequence;
and

5 determining from said intensity an extent of matching
 between said probe and said target sequence

wherein said method is a homogeneous assay conducted without providing a signal quenching agent on said target sequence or on said probe.

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